A. FRONT COVER/TITLE PAGE

TITLE OF RESEARCH PROJECT:

EFFECTS OF QUALITY COMPOSTS AND OTHER ORGANIC AMENDMENTS AND THEIR HUMIC AND FULVIC ACID FRACTIONS ON THE GERMINATION AND EARLY GROWTH OF SLICKSPOT PEPPERGRASS (*LEPIDIUM PAPILLIFERUM*) AND SWITCHGRASS IN VARIOUS EXPERIMENTAL CONDITIONS

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NAME OF CONTRACTOR: UNIVERSITA' DI BARI

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2nd INTERIM REPORT

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STATEMENT:
"THE RESEARCH REPORTED IN THIS DOCUMENT HAS BEEN MADE POSSIBLE THROUGH THE SUPPORT AND SPONSORSHIP OF THE U.S. GOVERNMENT THROUGH ITS EUROPEAN RESEARCH OFFICE OF THE U.S. ARMY. THIS REPORT IS INTENDED ONLY FOR THE INTERNAL MANAGEMENT USE OF THE CONTRACTOR AND U.S. GOVERNEMENT"

END OF FRONT COVER/TITLE PAGE

Report Documentation Page

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4. TITLE AND SUBTITLE
Effects of Quality Composts and Other Organic Amendments and Their Humic and Fulvic Acid Fractions on the Germination and Early Growth of Slickspot Peppergrass (Lepidium Papilliferum) and Switchgrass in Various Experimental Conditions

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B. BODY OF THE REPORT

(1) SCIENTIFIC WORK DONE DURING THE REPORTING PERIOD

1. Preliminary experiments aiming to improve the germination of slickspot peppergrass (*Lepidium papilliferum*) seeds

1.1. Experimental

At the beginning of the second six-month period of this research, another series of experiments were performed in order to improve the germination of the native American species slickspot peppergrass (*Lepidium papilliferum*). All the germination trials carried out during the first six months of this Project, which results were shown and described in the first report, produced a quite unsatisfactory germination performance.

In these new experiments, sixty (60) seeds of slickspot peppergrass for each treatment were previously scraped dry with fine sand paper, soaked in distilled water for 12 h, and pierced with steel needle before starting germination experiments. Then, the seeds were placed in three Petri dishes (20 seeds for each dish) on filter paper and added with Nitch nutrient solution either alone (control) or containing also each of three humic acid-like (HAL) fractions previously isolated from three selected composts (a green compost (GC-HAL), a mixed compost (MC-HAL) and a green coffee compost (GCC-HAL)) at concentrations of 10, 50 and 200 mg/L. The main physical and chemical properties of the three compost samples and their HAL fractions were shown and described in detail in the previous (first) report of this Project and summarized in Table 1.

For the preparation of the three HAL fraction suspensions, each HAL fraction was first dissolved in few drops of KOH 0.1 M and then brought to the final volume with bidistilled water reaching a final pH ranging from 6.5 to 6.8. The Petri dishes containing the seeds were placed in a Fitotron growth chamber for 49 days under diffuse light of tungsten-lamps and constant temperature of 23°C. In this preliminary germination experiment, after each week, germinated seeds were counted.

In order to start early-growth experiments, all germinated seeds obtained in these preliminary experiments were transplanted in plastic pots containing Nitch nutrient solution and placed in the same chamber at the following conditions: (a) photoperiod of

12-h; (b) temperature of 23 °C during the illumination period and 18 °C during the dark period; and (c) constant humidity of 65%.

1.2. Results and Discussion

The effect of HALs at the three concentrations on seed germination in preliminary experiments is reported in Table 2. On a total number of 60 seeds for each treatment and the control, after 49 days germination percentages of about 50% or less were obtained for all treatments. Germination results were not very different for the three HAL fractions and the control, especially at concentration of 50 mg/L which produced the best germination results for any HAL (Table 2). All preliminary early-growth experiments conducted with seedlings coming from the above germination experiments were unsuccessful because in few days all seedlings died.

2. Germination and early-growth experiments of slickspot peppergrass (*Lepidium papilliferum*) seeds

2.1. Experimental

2.1.1. Germination

After the preliminary germination experiments described in the previous section, during the following months of this research period, a series of experiments were performed on the germination and early-growth of slickspot peppergrass in the presence of the same HALs at concentrations of 10, 50 and 200 mg/L. With respect to preliminary experiments, some changes were made in these experiments with the objective to enhance the germination percentage even more.

The treatments were prepared by adding 3 g of agar in 200 mL of Nitch nutrient solution alone (control) or in the presence of 10, 50 and 200 mg/L of each HAL fraction. The pH of all suspensions was adjusted at a value of 7.3 with a solution of KOH 0.1 M, and then they were sterilized at 120 °C for 15 minutes. After cooling, at room temperature conditions, until about 55 °C, the substrate were poured in Petri dishes where they were let to completely cool and solidify at room temperature. Twenty (20) seeds of slickspot peppergrass pretreated as described in section *1.1.* were placed in Petri dishes containing the various substrates (Fig. 1), and kept in a thermostated chamber at the following conditions: (a) photoperiod of 12-h; (b) temperature of 18 °C during the illumination period and 10 °C during the dark period. After 7 days,

germinated seeds were counted and primary root and shoot lengths were measured. All experiments were triplicated and data obtained were statistically analyzed by one-way analysis of variance (ANOVA) at 95% and 99% confidence levels, and the mean values obtained for the different treatments were separated by using the least significant difference test (LSD).

2.1.2. Early-growth

The early-growth experiments were started on germinated seeds (seedlings) of slickspot peppergrass collected from the Petri dishes immediately after the end of the germination trials described in section *2.1.1*. The seedlings were transplanted into glass pots containing the same treatments described for germination experiments in section 2.1.1. i.e., Nitch nutrient solution in agar in the absence (control) or presence of each HAL at concentrations of 10, 50, and 200 mg/L.

The pots were coated with an aluminium foil to prevent root illumination except above where Parafilm was used to close the pots which were enough high to allow an adequate volume of air inside the pot thus avoiding oxygen limitations to seedlings. The pots were then placed in a thermostated chamber where seedlings were allowed to grow for a period of 35 days in the following conditions: (a) photoperiod of 12-h; (b) temperature of 18 °C during the illumination period and 10 °C during the dark period; and (b) constant humidity of 65%.

At the end of the experiment, the length of roots and shoots were measured. Because of the very little amount of plant material obtained, the fresh and dry weights of shoots and roots could not be measured. All experiments were conducted in three replicates and data obtained were statistically analyzed by ANOVA at 95% and 99% confidence levels, and the mean values obtained for the different treatments were separated by using the least significant difference test (LSD).

2.2. Results and Discussion

2.2.1. Germination

Numerical data of the effects of the three HALs examined at three different concentrations on the germination parameters of slickspot peppergrass are shown in Table 3 (absolute germination percentage) and Table 4 (absolute length, in cm, of primary shoot and root of germinated seeds) and Figs. 2 and 3 (same parameters expressed as percentages of those of the control treatment, assumed 100%).

The germination procedure described in section *2.1.1.* determined a marked increase of the germination percentage of slickspot peppergrass with respect to the procedure adopted previously and reported in section *1.1.* After 7 days, the percentage of germination ranged from a minimum of 56.7% in the treatment MC-HAL at 10 mg/L to a maximum of 85% in the treatment GC-HAL at 200 mg/L (Table 3 and Figs. 2 and 3). One-way Analysis of Variance of germination data showed the existence of a significant difference (0.05 P) only for the treatment GC-HAL at 200 mg/L with respect to the control (Fig. 2). Numerical data in Table 4 and Fig. 3 suggest that the three HALs at the three concentrations exerted a general slight positive effect in promoting primary shoot and root growth, with respect to the control. The stimulating effect, however, resulted statistically significant only for GC-HAL at 50 mg/L (0.05 P) and 200 mg/L (0.01 P), with an increase of primary shoot growth of 125 and 138%, respectively.

2.2.2. Early-growth

Numerical data of the effects of the three HALs examined at three different concentrations on the growth of shoots and roots of slickspot peppergrass measured after 35-day growth are shown in Table 5 (absolute length, in cm, of shoots and roots) and Fig. 4 (same parameters expressed as percentages of those of the control treatment, assumed 100%). Statistical treatment of data did not show significant difference between any treatment and the control.

3. Relationship between germination and early-growth data obtained and some compositional, structural and functional characteristics of HAL fractions

3.1. Experimental

The mean values obtained for the germination percentage, the lengths of primary root and shoot, and 35-day grown roots and shoots were statistically correlated to some chemical and functional properties (in Table 1) of the HALs examined, including ash content, acidic functional groups, E_4/E_6 ratio, and relative fluorescence intensity.

3.2. Results and discussion

The correlation coefficients calculated between some HAL properties and the average of variations (%) of germination percentage, lengths of primary root and shoot and roots and shoots of plants grown for 35 days are reported in Tables 6 and 7. In the case of

germination parameters, significant negative correlations were apparent between: (a) ash content and primary root length at 10 mg/L ($P \leq 0.05$); (b) phenolic OH and the germination percentage at 10 mg/L ($P \leq 0.01$); (c) E_4/E_6 ratio and primary root length at 50 mg/L ($P \leq 0.01$), whereas the only significant positive correlation was apparent between COOH group content and primary root length at 10 mg/L ($P \leq 0.05$) (Table 6). In the case of growth parameters, significant negative correlations were apparent between: (a) ash content and shoot length at 10 mg/L ($P \leq 0.05$); (b) E_4/E_6 ratio and shoot length at 50 mg/L ($P \leq 0.05$) and root length at 200 mg/L ($P \leq 0.01$); whereas the only significant positive correlation was apparent between COOH group content and shoot length at 10 mg/L ($P \leq 0.05$) (Table 7).

(2) RESEARCH PLANS FOR REMAINDER OF THE CONTRACT PERIOD

For the first 6 months of the second financed year of the contract period, research plans are the following:

(a) Experiments on germination and early growth of pre-treated peppergrass seeds in the presence of each compost and their DOM samples at various concentrations.
(b) Correlation of the germination and seedling growth data obtained with chemical and physico-chemical parameters of the composts and DOMs examined, in order to find out the parameters that influence germination and growth of peppergrass.

(3) SIGNIFICANT ADMINISTRATIVE ACTIONS DURING THE PERIOD REPORTED: NONE.

(4) ANY OTHER INFORMATION : NONE.

(5) ANNEX

(A) AMOUNT OF UNUSED FUNDS REMAINING ON THE CONTRACT AT THE END OF THE PERIOD COVERED BY THE REPORT: NONE
(B) IMPORTANT PROPERTIES ACQUIRED WITH CONTRACT DURING THIS PERIOD: NONE.
(C) METHOD OF REPRODUCTION: E-MAIL ATTACHMENTS, PHOTOCOPYING.

Table 1. Ash and acidic functional group content (on moisture free basis), E_4/E_6 ratio, and relative fluorescence intensity of HALs examined.

Origin of HAL	Ash %	COOH meq g^{-1}	Phenolic OH meq g^{-1}	Total acidity meq g^{-1}	E_4/E_6 ratio	RFI
GC	3.44	3.34	1.80	5.14	8.24	50.4
MC	3.21	3.38	2.94	6.32	8.08	46.9
GCC	4.96	3.08	2.55	5.63	8.54	36.9

Table 2. Effect of HALs at different concentrations on seed germination (number of germinated seeds on a total number of 60 seeds) of slickspot peppergrass after different periods.

Treatment		Number of germinated seeds							
		Days							
		7	14	21	28	35	42	49	**Total**
Control (Nitch nutrient solution)		5	5	0	0	6	14	0	**30**
GC-HAL	10 mg/L	5	3	0	0	6	6	2	**22**
	50 mg/L	1	4	5	3	7	8	1	**29**
	200 mg/L	3	4	2	1	2	11	2	**25**
MC-HAL	10 mg/L	5	5	0	3	0	7	1	**21**
	50 mg/L	9	8	0	2	2	8	3	**32**
	200 mg/L	4	4	0	2	1	16	0	**27**
GCC-HAL	10 mg/L	5	4	1	4	2	14	0	**30**
	50 mg/L	3	6	5	4	5	4	4	**31**
	200 mg/L	7	3	0	2	1	10	1	**24**

Table 3. Effect of HALs at different concentrations on seed germination (percentage of germinated seeds ± standard error for three replicates) of slickspot peppergrass measured immediately before transplanting.

Treatment	
Control (Nitch nutrient solution)	63.3 ± 7.58
GC-HAL 10 mg/L	75.0 ± 4.08
50 mg/L	70.0 ± 4.71
200 mg/L	85.0 ± 2.36
MC-HAL 10 mg/L	56.7 ± 8.92
50 mg/L	70.0 ± 8.16
200 mg/L	73.3 ± 5.44
GCC-HAL 10 mg/L	63.3 ± 8.28
50 mg/L	75.0 ± 6.24
200 mg/L	65.0 ± 6.24

Table 4. Effect of HALs at different concentrations on the length (cm ± standard error for three replicates) of primary shoot and primary root of germinated seeds of slickspot peppergrass.

Treatment	**Shoot**	**Root**
Control (Nitch nutrient solution)	0.77 ± 0.51	0.93 ± 0.74
GC-HAL 10 mg/L	0.86 ± 0.62	1.10 ± 0.17
50 mg/L	0.96 ± 0.45	1.12 ± 0.91
200 mg/L	1.06 ± 0.27	1.04 ± 0.74
MC-HAL 10 mg/L	0.84 ± 0.21	1.14 ± 0.81
50 mg/L	0.88 ± 0.44	1.19 ± 1.83
200 mg/L	0.89 ± 0.21	1.09 ± 0.14
GCC-HAL 10 mg/L	0.85 ± 0.33	0.91 ± 0.57
50 mg/L	0.89 ± 0.42	0.97 ± 0.17
200 mg/L	0.86 ± 0.56	0.90 ± 0.65

Table 5. Effect of HALs at different concentrations on the length (cm ± standard error for three replicates) of shoots and roots of slickspot peppergrass measured after 35-day growth.

Treatment		**Shoot**	**Root**
Control (Nitch nutrient solution)		1.16 ± 1.04	1.92 ± 2.62
GC-HAL	10 mg/L	1.06 ± 1.00	1.86 ± 1.08
	50 mg/L	1.21 ± 0.85	2.23 ± 0.59
	200 mg/L	1.23 ± 0.97	1.48 ± 2.02
MC-HAL	10 mg/L	1.09 ± 1.52	2.05 ± 1.48
	50 mg/L	1.38 ± 0.35	1.81 ± 3.76
	200 mg/L	1.15 ± 1.48	1.74 ± 3.58
GCC-HAL	10 mg/L	0.94 ± 1.39	1.24 ± 2.95
	50 mg/L	0.95 ± 0.87	1.49 ± 4.17
	200 mg/L	1.21 ± 0.26	0.98 ± 1.54

Table 6. Correlation coefficients calculated between germination parameters of slickspot peppergrass, expressed as the absolute average of variations (%), and some properties of HALs.

HAL properties [b]	**Average** [a]			**HAL 10 mg/L**			**HAL 50 mg/L**			**HAL 200 mg/L**		
	Primary shoot	Primary root	Germinated seeds (%)	Primary shoot	Primary root	Germinated seeds (%)	Primary shoot	Primary root	Germinated seeds (%)	Primary shoot	Primary root	Germinated seeds (%)
Ash (%)		--- [c]			**-0.999 ***			--	+++		---	
COOH (meq/g)		+++ [d]			**0.999 ***			++	---		+++	
Phenolic OH (meq/g)	-		--			**-1.000** **	--					
Total acidity (meq/g)				--		--						
E_4/E_6		---			--			**-1.000 ****	+		---	
IF					+				--			+

[a] Average of variations (%) obtained at 10, 50 and 200 mg/L
[b] Data in Table 1
[c] Negative correlation coefficient: (-) absolute value > 0.90, (--) > 0.95, and (---) > 0.99
[d] Positive correlation coefficient: (+) > 0.90, (++) > 0.95, and (+++) > 0.99
* Statistically significant at $P \leq 0.05$
** Statistically significant at $P \leq 0.01$

Table 7. Correlation coefficients calculated between growth parameters of slickspot peppergrass measured after 35 days, expressed as the absolute average of variations (%), and some properties of HALs.

HAL properties [b]	**Average** [a]		**HAL 10 mg/L**		**HAL 50 mg/L**		**HAL 200 mg/L**	
	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots
Ash (%)	---	---	**-0.999 ***	---	--			--
COOH (meq/g)	+++	+++	**0.999 ***	+++	++			++
Phenolic OH (meq/g)								
Total acidity (meq/g)							--	
E_4/E_6	---	-	--	---	**-0.998 ***			**-1.000 ****
IF		++	+			+		

[a] Average of variations (%) obtained at 10, 50 and 200 mg/L
[b] Data in Table 1
[c] Negative correlation coefficient: (-) absolute value > 0.90, (--) > 0.95, and (---) > 0.99
[d] Positive correlation coefficient: (+) > 0.90, (++) > 0.95, and (+++) > 0.99
* Statistically significant at $P \leq 0.05$
** Statistically significant at $P \leq 0.01$

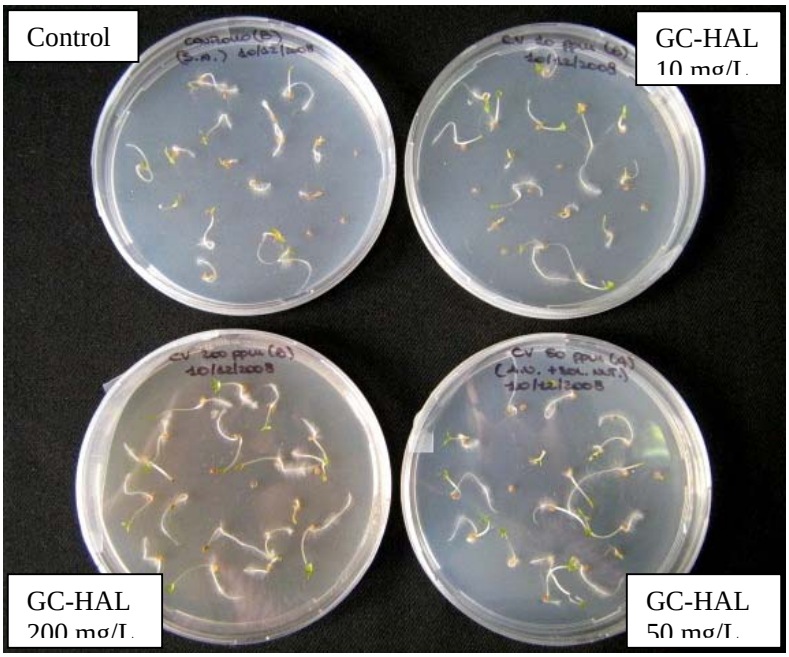
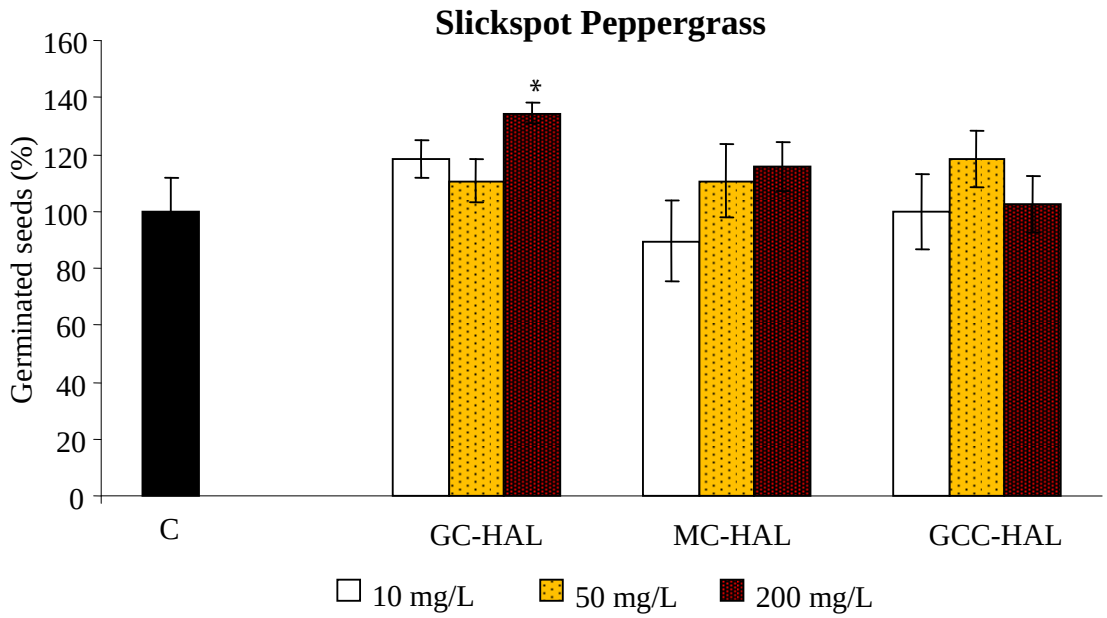
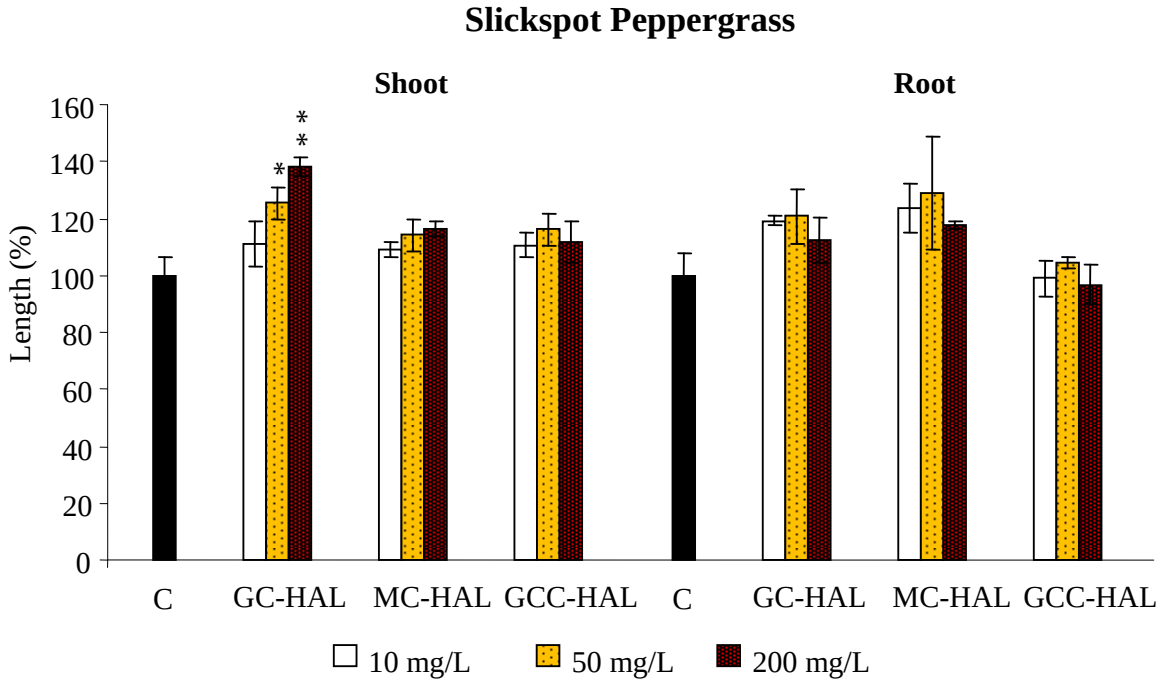


Figure 1. Effect of GC-HAL at different concentrations on the germination of slickspot peppergrass in agar substrate.



* P ≤ 0.05, according to the LSD test

Figure 2. Effect of HALs at different concentrations on the number of germinated seeds expressed as percentages of control treatment (100 %). The vertical line on each bar indicates the standard error for 3 replicates.



* P ≤ 0.05; **P ≤ 0.01, according to the LSD test.

Figure 3. Effect of HALs at different concentrations on primary shoot and root length of germinated seeds, expressed as percentages of control treatment (100 %). The vertical line on each bar indicates the standard error for 3 replicates.

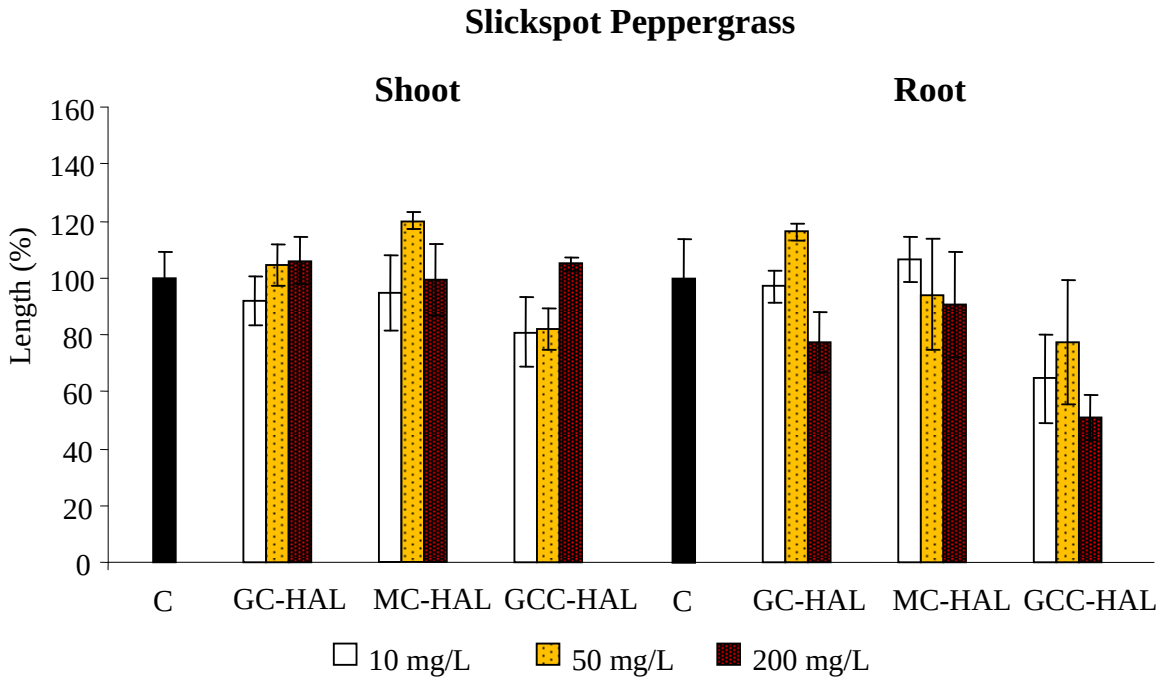


Figure 4. Effect of HALs at different concentrations on the length of shoots and roots expressed as percentages of control treatment (100 %) measured after 35-day growth. The vertical line on each bar indicates the standard error for three replicates.